Intrinsic Frequency Tuning in ELL Pyramidal Cells Varies Across Electrosonory Maps

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Mehaffey WH, Maler L, Turner RW. Intrinsic frequency tuning in ELL pyramidal cells varies across electrosonory maps. J Neurophysiol 99: 2641–2655, 2008. First published March 26, 2008; doi:10.1152/jn.00028.2008. The tuning of neuronal responsiveness to specific stimulus frequencies is an important computation across many sensory modalities. The weakly electric fish Apteronotus leptorhynchus detects amplitude modulations of a self-generated quasi-sinusoidal electric organ discharge to sense its environment. These fish have to parse a complicated electrosonory environment with a wide range of possible frequency content. One solution has been to create multiple representations of the sensory input across distinct maps in the electrosonory lateral line lobe (ELL) that participate in distinct behavioral functions. E- and I-type pyramidal cells in the ELL that process sensory input further exhibit a preferred range of stimulus frequencies in relation to the different behaviors and sensory maps. We tested the hypothesis that variations in the intrinsic spiking mechanism of E- and I-type pyramidal cells contribute to map-specific frequency tuning. We find that E-cells exhibit a systematic change in their intrinsic spike characteristics and frequency tuning across sensory maps, whereas I-cells are constant in both spike characteristics and frequency tuning. As frequency tuning becomes more high-pass in E-cells, the refractory variables of spike half-width and afterhyper-polarization magnitude increase, spike threshold increases, adaptation becomes faster, and the gain of the spiking response decreases. These findings indicate that frequency tuning across sensory maps in the ELL is supported by differences in the intrinsic spike characteristics of pyramidal cells, revealing a link between cellular biophysical properties and signal processing in sensory maps with defined behavioral roles.

INTRODUCTION

The complex and multi-dimensional nature of sensory stimuli often contains more information than a single neuron can accurately represent in a spike train. For the nervous system to encode important characteristics of the outside world, sensory neurons must initially respond to specific aspects of the sensory input. One component of natural signals commonly encoded by sensory neurons is frequency (either spatial or temporal), which has been shown to be important for visual (Butts et al. 2007; Lesica and Stanley 2004), auditory (Fritz et al. 2003; Woolley et al. 2005), somatosensory (Luna et al. 2005; Romo et al. 2004), and electrosonory (Chacron et al. 2003; Gabbiani and Metzner 1999; Oswald et al. 2004; Shumway 1989) processing. The ability of a cell to respond to specific frequencies within a broadband signal implies mechanisms for frequency tuning. Such frequency tuning can be implemented by intrinsic conductances and cell dynamics (Ellis et al. 2007b; Izhikevich 2002), by feedforward synaptic dynamics (Zhang et al. 2003), by short-term synaptic plasticity (de la Rocha and Parga 2005), and by feedback from higher brain centers (Chacron et al. 2003, 2005).

The electric fish Aperonotus leptorhynchus generates a weak quasi-sinusoidal electric organ discharge (EOD). Amplitude modulations (AMs) generated by perturbations of the field created by the EOD are used to sense the environment, to establish social hierarchies, and to communicate with conspecifics (Heiligenberg 1991). The electrosonory lateral line lobe (ELL) is the first CNS stage of electrosonory processing in the medulla of gymnotiform fish. It is divided into four topographic maps of the body surface termed the medial (MS), centromedial (CMS), centrolateral (CLS), and lateral (LS) segments (Bell and Maler 2005). Pyramidal neurons of the CMS, CLS, and LS receive primary afferent input from tuberosous receptors that detect perturbations in the amplitude of the EOD. On entering the ELL, primary afferent fibers trifurcate and provide identical tuberosous receptor input to each of the three maps (Carr et al. 1982; Heiligenberg 1991). The neural circuits in each of these three segments are also morphologically similar and function essentially as repeating topographical maps with no inter-map connections. The formation of distinct maps within a sensory modality is common in sensory systems and has been shown to allow parallel processing of different types of information (Metzner 1999; Schreiner and Winer 2007; Young 1998). In the ELL, pyramidal cells within these three segments show different preferences for the frequency content of input signals. Recordings in vivo reveal that cells in the more medial segments prefer inputs at lower frequencies while the more lateral segments prefer higher frequencies (R. Krahe, personal communication) (Shumway 1989). Furthermore, the differential frequency tuning between maps correlates to specific electrosonory behaviors: more medial segments process low-frequency-related input associated with a jamming avoidance response, and lateral segments process the high-frequency inputs involved in communication (Metzner and Jurane 1997). As ELL pyramidal cells show frequency selectivity to broadband inputs both in vitro (Ellis et al. 2007b) and in vivo (Chacron et al. 2003, 2005), they present an ideal model to identify the cellular and synaptic basis of frequency tuning across multiple sensory maps in the CNS.

Pyramidal cells within the ELL maps can be subdivided into two types based on the presence or absence of a basilar
dendrite. Basilar pyramidal cells (E-cells) receive direct excitatory input from electroreceptive afferents, whereas nonbasilar pyramidal cells (I-cells) are inhibited by granule cell interneurons (GC2) activated by primary afferents. This architecture allows these two cell classes to respond preferentially to upstrokes (E-cells) and downstrokes (I-cells) of EOD AMs as they are excited or inhibited by increases in EOD amplitude in the intact animal (Heiligenberg 1991). In vivo recordings have established a further frequency preference between these cells in the CLS and LS maps, with E-cells exhibiting tuning to higher frequencies and exhibiting greater regulation of frequency tuning than I-cells (Chacron et al. 2005). These results are also consistent with observations in vitro that intrinsic membrane properties of the E- and I-cell class are biased toward different frequency ranges (Ellis et al. 2007b). However, the contribution of intrinsic cellular mechanisms to differential frequency tuning across ELL maps remains to be determined. One promising line of investigation has examined the detection of input frequency by bursts of spikes. Indeed the segregation of bursts and single spikes to encode distinct frequency ranges has been observed in electric fish (Doiron et al. 2007; Gabbiani et al. 1996; Oswald et al. 2004, 2007) and in the mammalian visual (Lesica and Stanley 2004; Lesica et al. 2006) and auditory systems (Eggermont and Smith 1996). Bursts have also been shown to be more reliably timed to stimulus features, to show superior feature detection properties, and to more reliably activate downstream cells than can single spikes (Gabbiani et al. 1996, Izhikevich et al. 2003; Kepecs and Lisman 2003; Lisman 1997; Metzner et al. 1998). This suggests that bursts may have specific functions in information coding.

Extensive work on ELL pyramidal cells in vitro and in vivo has identified the role and significance of an intrinsic somadendritic interaction involving a conditional backpropagation of dendritic spikes in the generation of burst discharge (Turner et al. 1994). Moreover, this work has shown that pyramidal cell bursts are important for feature detection of sensory stimuli and particularly for low-frequency components of a stimulus (Mehaffey et al. 2007; Metzner et al. 1998; Oswald et al. 2004, 2007). The ability of pyramidal cells to encode low-frequency events with bursts is under significant regulation by intrinsic conductances (Ellis et al. 2007b; Rashid et al. 2001b), synaptic input (Mehaffey et al. 2007), and neuromodulatory agents (Ellis et al. 2007a) that can affect the underlying soma-dendritic interaction. Indeed we have previously shown that burst discharge and frequency tuning can be affected by the properties of pyramidal cell spike discharge. For instance, a high-threshold potassium current expressed in pyramidal cells allows high frequencies of spike discharge (Fernandez et al. 2005a). Increases in somatic spike width can alter bursting by reducing the mismatch between somatic and dendritic voltage that underlies a depolarizing afterpotential involved in producing spike bursts (Fernandez et al. 2005b). An apamin-sensitive AHP is involved in creating the frequency tuning profile of pyramidal cells (Ellis et al. 2007b), while a theoretical study has further shown that the adaptation rate of pyramidal cell discharge is capable of regulating low-frequency tuning (Benda and Herz 2003). Inasmuch as these parameters of spike discharge could be regulated in pyramidal cells, they could contribute to establishing differential frequency tuning across sensory maps.

In the present study, we tested the hypothesis that differential frequency tuning by pyramidal cells across ELL sensory maps involves the regulation of membrane properties that underlie spike generation and bursting, and their adaptation rates in response to quasi-naturalistic stimuli. In fact, we find that several fundamental aspects of ELL pyramidal cell spike waveform differ across maps in a manner that can contribute to differential frequency tuning.

**METHODS**

**Preparation of slices for electrophysiology**

*A. leptorhynchus* were obtained from local importers and maintained at 26–28°C in fresh water aquaria in accordance with protocols approved by the University of Calgary Animal Care Committee. All chemicals were obtained from Sigma (St. Louis, MO) unless otherwise noted. Animals were anesthetized in 0.05% phenoxy-ethanol, and ELL tissue slices of 300–400 μm thickness were prepared as previously described (Turner et al. 1994). Slices were maintained by constant perfusion of ACSF (1–2 ml/min) and superfusion of humidified 95% O2-5% CO2 gas. ACSF contained (in mM) 124 NaCl, 3 KCl, 25 NaHCO3, 1.0 CaCl2, 1.5 MgSO4, and 25 D-glucose, pH 7.4. HEPES-buffered ACSF for pressure ejection of pharmacological agents contained the same elements with the following differences: 148 mM NaCl, 10 mM HEPES.

**Recording procedures**

Glass microelectrodes were backfilled with 2 M KAc (pH 7.4; 90–120 MΩ resistance) containing 2% Neurobiotin (Vector Labs, Burlington, ON, Canada). A total of 118 recordings were obtained from pyramidal cells in all three ELL segments receiving tuberous inputs (sample sizes for CMS: 44, CLS: 47, LS: 27). The map in which recordings were made was identifiable as the borders between maps are clearly defined in the in vitro slice. Recordings were digitized at 10–40 kHz using a NI PCI-6030E DAQ board (National Instruments, Austin, TX) and recorded in custom software using the Matlab data-acquisition toolbox (Mathworks, Natick, MA). Cells were held at a level just below firing threshold using negative current injection. Random amplitude modulations (RAMs) consisting of white noise low-pass filtered to 0–60 Hz were provided on top of the bias current and the SD of the waveform was adjusted to give average firing rates of 10–30 Hz, as at these frequencies the estimated coherences were reliable in their frequency preferences. Recordings were rejected if any part of the stimulation protocol could not be performed.

**Cell fills**

Pyramidal cells were filled with Neurobiotin using a positive current ejection pulse (+1 nA, 2 Hz). Following recording slices were transferred to 4% paraformaldehyde and fixed for several days at 4°C. Slices were then washed in 0.1 M phosphate buffer (PB) for several hours and placed in a solution of PB, Triton X-100 (0.1%), DMSO (0.5%), and streptavidin-Cy3 (1:1500) for 3 days. Slices were slide-mounted for visualization on an Olympus BH-2 research microscope to identify filled cells according to E- or I-cell, position within the ELL pyramidal cell body layer (PCL, superficial, intermediate or deep), and segment (CMS, CLS, or LS). Images were taken using Fluoview software on an Olympus FV300 BX50 confocal microscope. Of 118 cells recorded from the three maps, 43 were successfully filled with Neurobiotin. Superficial pyramidal cells were distinguished by the extent of their dendritic arborization and by location of the soma within the cell layer (Bastian and Nguyenkim 2001). Cells were classified as E- or I-cells depending on the presence or absence
of a basilar dendrite or by their frequency preference (Ellis et al. 2007b). Of the filled cells classified following Neurobiotin injection, 8 were from the LS, 11 from the CLS, and 24 from the CMS.

Data analysis

All electrophysiological data were analyzed in Matlab. Data were plotted in Origin (OriginLab, Northampton, MA) or Igor Pro (Wave metrics, Lake Oswego, OR). Spike trains were digitized into binary trains in 0.5-ms bins and detrended to obtain a zero mean binary spike train. Coherence estimates were made between the binary spike trains and the original RAM stimulus and given by

\[ C(f) = \frac{P_u(f)^2}{P_u(f)P_v(f)} \]

where \( P_u \) and \( P_v \) denote the power spectrum of the stimulus and the response respectively, and \( P_u \) denotes the cross-spectrum between the stimulus and response (e.g., the spike train). Pyramidal cells displaying spontaneous slow oscillations (Turner et al. 1996) were excluded from analysis.

Spike characteristics were measured at the weakest magnitude of DC current injection that elicited spiking and analyzed with custom software in Matlab. Afterhyperpolarization (AHP) measurements were taken as the minimum voltage between spikes. Spike threshold was assessed based on the derivative of the voltage trace and defined as the initial value eight times greater than the SD of the subthreshold noise. The level of current injection required to elicit bursting was assessed by visual inspection. Burst threshold was then defined as the amount of current injection required to transition from initial tonic spiking into bursting (Mehaffey et al. 2007; Noonan et al. 2003). Cells were classified as nonbursting if they reached a saturating firing frequency but did not burst or if \( >0.6 \) nA of current was injected above spike threshold without eliciting bursting. Gain was determined from the slope of a linear fit to the FI curve (Mehaffey et al. 2005).

Adaptation time constants were fit to 100-s RAMs with spike trains binned into 1-s segments. Statistical significance was assessed using a one-way ANOVA with a significance level of \( P < 0.05 \) unless otherwise noted, with post hoc analysis using Tukey’s HSD. Average values are presented as means \( \pm \) SE.

Classification of cell types based on coherence

As we recorded primarily from the PCL, we sampled from populations of intermediate and superficial pyramidal cells and were unlikely to have recordings from deep pyramidal cells positioned adjacent to or within the granule cell layer (Bastian and Courtright 1991; Bastian et al. 2004). Further identification of pyramidal cell class on the basis of firing properties to square-wave current injection protocols has traditionally been difficult in vitro, requiring the filling and labeling of cells for histological analysis (Ellis et al. 2007b). In contrast, during in vivo recordings, the response to increases in EOD amplitude can be quickly tested and the cell identified as corresponding to either the E- or I-cell class. Our recent work established that a time-varying stimulus can also be used to distinguish E- and I-type pyramidal cells in vitro in terms of the frequency dependence of their response (Ellis et al. 2007b). Using this procedure, histologically identified I-cells in all segments were found to be ubiquitously low-pass in their frequency responsiveness, whereas identified E-cells in the CLS and LS were either broadband or high-pass in frequency responsiveness. In the CMS, superficial E-cells could deviate from this pattern in exhibiting low-pass responsiveness (Ellis et al. 2007b). Each of these findings were confirmed in the present study, with low-pass frequency responsiveness defined as coherence ratios \( [C(30–50 \text{ Hz})/C(0–20 \text{ Hz})] \) within 2 SDs above the average for anatomically identified I-cells (upper cut-off of 0.86). No anatomically filled E-cells in the CLS or LS fell into this range. Although this does not guarantee the absence of misclassifications, it suggests that such errors are rare.

The remaining cells were then subdivided into a class of broadband and high-pass cells by a coherence ratio of 0.87 to 1.19 (broadband), or a coherence ratio >1.19 (displaying an apparent preference for higher frequencies, see Fig. 6A and accompanying results). The E-cell group included any basilar pyramidal cells in the CMS that had been successfully labeled regardless of frequency preference, given the similarity in frequency tuning for some CMS E-cells (superficial pyramidal cells) with I-cells. Therefore our group of E-like CMS pyramidal cells is composed of intermediate basilar pyramidal cells along with superficial cells directly identified by intracellular fills. Further, our low-pass cell population may include a small number of superficial E-type CMS pyramidal cells that display low-pass characteristics (Ellis et al. 2007b). Although in the CMS this could lead to misclassification, any such errors would lead to the misclassification of superficial E-cells as I-cells. This would lead to an increase in the number of low-pass cells classified as I-cells, and a corresponding decrease in the number identified as E-cells. As no such differences were found, we are confident in our conclusion that although E- and I-cells of the CMS might show minor differences they are, as a population, indistinguishable.

RESULTS

One well-studied characteristic of ELL pyramidal cells is a specific pattern of burst firing, consisting of a gradually increasing rate of firing followed by a brief “doublet” of spikes with a short ISI (<10 ms) (Fernandez et al. 2005b; Lemon and Turner 2000; Turner et al. 1994) that can be observed both in vivo and in vitro (Oswald et al. 2004). We began by studying the distribution and characteristics of this burst discharge. During step depolarizations, one can detect a “slow burst dynamic” as a progressively increasing firing frequency terminated by a fast ISI (the doublet). This dynamic is driven by a frequency-dependent increase in the peak latency and duration of dendritic spikes that in turn augment dendro-somatic current flow to enhance a somatic depolarizing afterpotential (DAP) (Fernandez et al. 2005b). The short ISI of the spike doublet terminates the burst when it falls within the dendritic refractory period to block dendritic backpropagation. During in vitro recording, we observed burst firing in pyramidal cells of all three tuberous maps, with representative examples of burst discharge from each map shown in Fig. 1C in response to step current pulses. We were able to classify E- or I-cells in the CLS and LS using a recently established methodology based on their response to broadband inputs (Ellis et al. 2007b), and through intracellular labeling (CMS: 23, CLS: 11, LS: 8). Specifically, the stimulus-response coherence shows a peak at low frequencies in I-cells, whereas E-cells rarely show a preference for low frequencies. We thus used this distinction to classify cells when intracellular labeling was unsuccessful (see METHODS) and were able to identify unlabeled I-cells in each of the three maps (CMS: 35, CLS: 31, LS: 13) as well as E-cells (CMS: 9, CLS: 16, LS: 14) by these criteria.

Expression of burst discharge in response to DC steps across tuberous maps and cell types

We found significant differences in the fraction of E- and I-cells that displayed burst firing in each of the tuberous maps (Kruskal-Wallis 1-way ANOVA, \( P < 0.05 \)). I-cells showed the greatest propensity to burst with the majority of I-cells in each...
map displaying bursting (CMS: 86%, CLS: 82%, LS: 70%; Fig. 1D). In contrast, bursting was less prevalent in E-cells in all maps with the smallest proportion of bursting cells apparent in the LS (CMS: 22%, CLS: 25%, LS: 7%; Fig. 1D). The incidence of bursting in E-cells proved to be significantly lower than that of I-cells regardless of map (P < 0.05, Mann-Whitney U). We then proceeded to examine the rheobase for burst firing, taken as the difference between the DC current injection required to evoke a burst and that required to evoke initial tonic spiking. Burst rheobase was characterized across maps exclusively for I-cells as the low number of E-cells that displayed bursting precluded any significant statistical power. Burst rheobase was almost identical between I-cells of the CMS and CLS (0.2 ± 0.02 and 0.2 ± 0.03 nA, respectively, P > 0.05) but was significantly lower in the LS (0.09 ± 0.01 nA, P < 0.05) in comparison to the other two maps (Fig. 1E).
E-cells as a population were not significantly different from the population of I-cells (E-cells, 0.25 ± 0.07 nA, I-cells, 0.21 ± 0.03, P > 0.05). As ELL pyramidal cells appear to rest at membrane potentials near spike threshold, these results suggest that LS I-cells in vivo will have a lower threshold for bursting relative to I-cells in the other maps, although adaptation, threshold, differences in the convergence of synaptic input, and other processes (see following text) may also have an influence in vivo.

Expression of burst discharge in response to time-varying inputs

We extended our examination of burst firing by characterizing the response to time-varying inputs that more accurately simulate the input pyramidal cells receive in vivo. It has been established previously that pyramidal cell membrane voltage can accurately encode 0- to 60-Hz RAMs presented in vivo (Chacron et al. 2003; Middleton et al. 2006). We have therefore been able to use intracellular RAM current injection in vitro to mimic driving currents that result from external field RAMs in vivo (Ellis et al. 2007b; Mehaffey et al. 2007; Oswald et al. 2004, 2007). Briefly, we adjust the amplitude of a 100-s, 0- to 60-Hz RAM intracellular current stimulus to give an average 10- to 30-Hz firing rate, similar to the firing frequencies recorded in vivo during external RAM stimuli (Chacron et al. 2003, 2005; Doiron et al. 2003a).

Bursts evoked by time-varying inputs require the positive dendro-somatic feedback known to be generated by backpropagating apical dendritic spikes (Oswald et al. 2004). In response to RAMs, bursts are more often truncated to spike doublets, either through dendritic failure or rapid hyperpolarizations induced by high-frequency variations of the signal (Doiron et al. 2007). In fact, the dendrosomatic feedback is present even in cells that do not undergo the slow burst dynamic in response to DC current steps (Mehaffey et al. 2005). As a result of this spike-dependent feedback, time-varying inputs invoke a bimodal ISI histogram that can be divided into burst events and isolated spikes regardless of their ability to burst in response to DC current injections. We therefore parsed the resulting spike trains into bursts (typically spike doublets) and isolated spikes and separately computed the stimulus-response coherence for these events (Fig. 2).

It has been shown that bursts of spikes code preferentially for low-frequency events, while isolated spikes show a broadband or high-pass frequency preference (Mehaffey et al. 2007; Oswald et al. 2004). The selective encoding of low-frequency inputs by bursts of spikes both requires and is regulated by the intrinsic burst mechanism (Ellis et al. 2007b; Mehaffey et al. 2007; Oswald et al. 2004). To examine the relationship be-

FIG. 2. Response of pyramidal cells to time-varying inputs. A: representative recording (top) from an I-cell of the CLS showing the patterns of bursts (↓) and isolated spikes evoked in response to a time-varying intracellular current injection [random AM (RAM) bottom trace]. B: ratio of burst events in comparison to all spike events (burst fraction) evoked in response to time-varying stimuli. The total number of observed bursts decreases from medial to lateral segments in E-cells but not I-cells. C: fraction of the low-frequency (0–20 Hz) coherence encoded by bursts. A large fraction is encoded in all I-cells and CMS E-cells, but a smaller fraction is encoded by CLS and LS E-cells. D and E: representative interspike interval (ISI) densities for I-cells (D) and E-cells (E). Across maps, the timing of the nadir of the bimodal ISI histograms is conserved. Further, the number of events classifiable as burst events (e.g., the left peak) decreases from CMS to LS segments. ↓, the nadir of the histogram used to distinguish short ISIs (e.g., burst events).

J Neurophysiol • VOL 99 • MAY 2008 • www.jn.org
between low-frequency events and bursts of spikes, we quantified the fraction of the total 0- to 20-Hz coherence encoded by bursts of spikes. We found that this measure closely followed the pattern observed in the burst fraction (Fig. 2B), where I-cells of all segments had the greatest ability to encode low-frequency events with bursts of spikes (CMS: 0.52 ± 0.03, CLS: 0.53 ± 0.04, LS: 0.53 ± 0.08, P > 0.05). E-cells of the CMS were also able to encode low-frequency events (CMS: 0.47 ± 0.04) at a level that was statistically indistinguishable from I-cells of all segments (Fig. 2C). In comparison, E-cells of the CLS and LS showed a reduced tendency to encode low-frequency inputs with bursts of spikes (CLS: 0.32 ± 0.04, LS: 0.24 ± 0.04, P < 0.05).

Taken together, these data suggest that the degree to which ELL pyramidal cells encode low frequencies with bursts varies across the maps (for E-cells) and cell types (E- vs. I-cells). The cells with the greatest tendency to express the slow burst dynamic (e.g., Fig. 1D) also show the strongest ability to encode low-frequency inputs using bursts. This occurs despite the fact that the positive dendro-somatic feedback required to establish the ISI histogram bimodality (Oswald et al. 2004) occurs even in cells that do not express these slow burst dynamics (Mehaffey et al. 2005). Although LS I-cells show a lower burst threshold in response to brief DC inputs, long-lasting RAMs invoked burst discharge no more often than in I-cells in other segments. This may be due to the adaptation observed over long periods of stimulation (see following text). Such adaptation implies that the lower threshold for bursting in LS I-cells would be observed primarily in response to low-frequency transients larger than the average background fluctuations to which they have adapted.

We plot representative ISI histograms for both I-cells and E-cells in Fig. 2, D and E, using a log scale for time to clearly show the bimodality (Ellis et al. 2007b; Turner et al. 1996). Previous studies have suggested that the nadir of the ISI histogram is highly conserved in the in vitro preparation (Ellis et al. 2007b; Mehaffey et al. 2007; Oswald et al. 2004; Turner et al. 1996). As described in the preceding text, all pyramidal cells, regardless of map or cell class, showed a clear bimodality with a nadir near 10 ms (8–10 ms, P > 0.05 across cell types and all maps), establishing ISIs < 10 ms as our burst ISI criterion. Note that the lower density of ISIs in the first peak of the ISI histogram in E-cells of the CLS and LS are indicative of the lower burst fraction observed in these cells. A previous study examined differences between maps and between E- and I-cells, reporting that I-cells were better feature detectors and that the CMS as a whole outperformed the LS at feature detection, particularly when bursts where analyzed (Metzner et al. 1998). As bursting is more common in both I cells, and in the CMS (for E cells), this may be related to these in vivo results, particularly as the frequency cutoff for their RAM stimuli was between 2 and 40 Hz, a frequency range where bursts are expected to play an important role (Doiron et al. 2007).

**E- and I-cells differ in spike properties**

We recently showed that the SK potassium channel mediated AHPs of pyramidal cells differ between E- and I-cells and across ELL maps and differentially regulate the frequency selectivity of cells (Ellis et al. 2007b). We thus tested the hypothesis that differences in burst output and frequency tuning across ELL maps reflect a different complement of ionic currents between E- and I-cells. We first grouped all the identified E-cells (n = 39) and I-cells (n = 79), and examined various parameters of evoked spikes to establish whether there were any significant differences between the two cell classes. We examined the average characteristics of the spike waveform at the minimal current injection sufficient to induce repetitive firing. E- and I-cells proved to be significantly different in all of three key parameters examined (Fig. 3, A–C), including the magnitude of the AHP (I-cells: 6.18 ± 0.33, E-cells: 7.42 ± 0.41 mV, P < 0.05), spike half-width (I-cells: 6.2 ± 0.019 ms, E-cells: 8.1 ± 0.03, P < 0.05), and the voltage threshold for spiking (I-cells: −66.57 ± 0.074 mV, E-cells: −62.89 ± 0.74 mV, P < 0.05). Interestingly, the differences in voltage threshold for spiking may optimize a pyramidal cell’s response to the inputs they receive. E-cells receive direct excitatory input from electroreceptors, whereas I-cells translate the EOD primarily through the removal of disynaptic inhibition. A lower spike threshold in I-cells may then contribute to higher sensitivity to afferent input. The factors determining threshold remain unknown but could involve low-threshold potassium currents known to be expressed in ELL pyramidal cells (Ellis et al. 2007a; Fernandez et al. 2005a; Mathiesen and Maler 1988; Mehaffey et al. 2006; Smith et al. 2006). The larger AHP in E-cells may be related to the recently established preferential expression of somatic SK2 potassium channels in this cell class (Ellis et al. 2007b).

**FIG. 3.** E- and I-cell populations are significantly different with respect to spike properties. On average, E-cells exhibit a significantly wider spike (A), a larger magnitude AHP (B), and a higher spike threshold (C) than I-cells. Sample sizes for A–C are shown in brackets in C.
Spike characteristics show only minor differences across maps

A number of studies have found differences in the expression pattern of specific potassium channels across the tuberous maps of the ELL, generally with an increasing intensity of expression along the mediolateral axis (Deng et al. 2005; Ellis et al. 2007b; Mehaffey et al. 2006; Rashid et al. 2001a; Smith et al. 2006). Gradients of ion channel expression in the auditory system have been shown to underlie differences in the spikes generated, which in turn can contribute to specialized neuronal computations (Brew and Forsythe 2005; Li et al. 2001; Parameshwaran et al. 2001; Rosenblatt et al. 1997; von Hehn et al. 2004). We therefore examined spiking characteristics to see if these gradients of channel expression translate into differences in the spike waveform, and in turn, frequency tuning across maps.

We predicted that spike parameters would vary between cell type and map as suggested by the distribution of potassium channels. Ideally these characteristics would, like coherence, provide an electrophysiological signature to identify cells without histological analysis. We began by examining I-cells (low-pass). Representative examples of spikes recorded from I-cells from each of the three maps are shown in Fig. 4A. When I-cell spikes are superimposed, little variability is detected (Fig. 4B) as supported by the lack of significant differences for various spike parameters between I-cells in each of the three maps. Spike half-width was nearly identical in I-cells between the three maps (CMS: 0.623 ± 0.035 ms, CLS: 0.613 ± 0.028 ms, LS: 0.617 ± 0.018 ms, P > 0.05), suggesting a similar net complement of sodium and potassium channels underlying the spike regardless of map. In support of this, there were no significant differences between I-cells of different maps in terms of the magnitude of the AHP (CMS: 5.70 ± 0.385 mV, CLS: 7.13 ± 0.78 mV, LS: 6.31 ± 1.36 mV, P > 0.05; Fig. 4D) or the spike voltage threshold (CMS: 67.5 ± 1.08 mV, CLS: 65.3 ± 1.15 mV, LS: −67.4 ± 2.02 mV, P > 0.05; Fig. 4F).

![Fig. 4. Spike parameters of I- and E-cells across tuberous segments. A: representative traces of individual spikes of I-cells recorded from each of the 3 tuberous segments, CMS (light gray), CLS (dark gray), and LS (black). B: superimposition of spikes shown in A reveals minimal variability in spike shape. C: average values from recordings in each map show no significant differences in I-cell spike half-width, AHP size, or spike threshold. D: representative traces of individual spikes recorded from E-cells within the segments, CMS (light gray), CLS (dark gray), and LS (black). E: superimposition of spikes shown in D reveals larger spike width of LS E-cells. F: spike half-width increases in a medio-lateral fashion and is significantly longer in the LS segment, whereas no significant differences in AHP size or spike threshold are found across maps. Sample sizes for recordings in each segment are shown in brackets.](http://jn.physiology.org)
This suggests that I-cells are highly homogenous in their spiking characteristics across the three maps. In contrast, our examination of E-cells (as defined by the coherence criteria) showed that spike half-width varied significantly across maps. E-cells in the LS had a wider spike half-width than those in either the CMS or CLS (Fig. 4, D and E); the latter were not significantly different from each other (CMS: 0.79 ± 0.028 ms, CLS: 0.74 ± 0.026 ms, LS: 0.96 ± 0.054 ms, P < 0.05). No such differences were found in E-cell AHP depths (CMS: 7.16 ± 0.75, CLS: 7.48 ± 0.64, LS: 7.45 ± 0.86, P > 0.05) or spike threshold (CMS: −63.4 ± 1.01 mV, CLS: −64.5 ± 1.28 mV, LS: −60.98 ± 1.06 mV, P > 0.05; Fig. 4E). A recent theoretical study (J. Middleton, personal communication) suggested a computational advantage if E-cells of the LS have a higher threshold than cells in the other segments. To test this, we pooled the CMS and CLS cells for comparison to LS pyramidal cell threshold. This proved to reveal a significant difference (CMS/CLS pooled: −64.2 ± 0.8 mV, LS: −60.98 ± 1.0 mV, P < 0.05), suggesting that an increased threshold is indeed specific to pyramidal cells in the LS map.

The expression of SK2 potassium channels varies in a map-specific fashion for E-cells but not for I-cells (Ellis et al. 2007b). Taken together, our results suggest that other currents may follow a similar pattern, preferentially regulating the width and threshold of the spike waveform in the most lateral segment, but in a fashion exclusive to E-cells. However, the lack of a graded difference across maps in such variables as AHP depth does not match the expression pattern observed in immunohistochemical studies where mediolateral gradients of expression were observed for many different channels (Deng et al. 2005; Ellis et al. 2007b; Mehaﬀey et al. 2006; Rashid et al. 2001a; Smith et al. 2006). We hypothesized that these distinctions could lead to some heterogeneity in the computations performed by E-cells.

Frequency tuning characteristics vary with cell class and across maps

Having examined spike parameters, tendency to burst, and the relationship between bursting and low-frequency tuning in pyramidal cells, we next considered the full spike train, including both bursts and isolated spikes. We calculated the stimulus-response coherence for cells in all three maps and for both E- and I-cells using an intracellular current injected 0- to 60-Hz RAM stimulus. Figure 5 shows Neurobiotin fills of representative cells from each map along with the associated 0–60 Hz coherence. Consistent with a previous study (Ellis et al. 2007b), labeled I-cells were low-pass regardless of map (Fig. 5A), and displayed a clear peak in their stimulus-response coherence at low frequencies (0–20 Hz). In comparison, E-cells identified by their basilar dendrites (Fig. 5B) exhibited peaks in the coherence at low frequencies (e.g., Fig. 5B, CMS), a broadband frequency response (e.g., B, CLS), or a preference for higher frequencies (B, LS). Because our previous work had established that CMS E-cells can contain at least two populations (1 low-pass and 1 without noticeable frequency preference) (Ellis et al. 2007b), we considered whether similar

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**FIG. 5.** Frequency selectivity varies with cell type and map. In each case, representative examples of the coherence between a pyramidal cell spike train and the intracellular current RAM stimulus (0–60 Hz) is shown along with a representative Neurobiotin-labeled pyramidal cell. Basilar dendrites of E-cells are denoted in (B) by arrowheads. A: I-cells exhibit low-pass characteristics in preferring low frequency (0–20 Hz) over higher frequency (30–50 Hz) inputs to a similar degree across all ELL maps. B: E-cells show a clear shift in frequency selectivity across maps. Observed frequency tuning for E-cells includes low-pass (CMS), broadband (CLS), and high-pass (LS) selectivities. Scale bars: A: CMS, CLS: 75 μm, LS: 80 μm. B: CMS 60 μm, CLS, LS: 75 μm.
heterogeneities might exist within the more lateral maps. We determined the coherence ratio for all cells (e.g., the ratio of the 30- to 50-Hz coherence to the 0- to 20-Hz coherence). This gave us an index of the frequency tuning characteristics of a cell, where high coherence ratios indicate high-pass characteristics, and numbers near unity indicate broadband characteristics (Ellis et al. 2007b; Mehaffey et al. 2007). Previous work has determined that the low-frequency tuned population of I-cells showed coherence ratios <0.86, and this cut-off was used to separate a low-frequency responsive population (mean coherence ratio, 0.66 ± 0.01). We then considered the possibility that cells may be subdivided into those displaying an opposite frequency preference—e.g., a preference for high frequencies. An examination of the distribution of coherence ratios of the cells (Fig. 6A) allowed a further separation into a population between 0.86 and 1.19 (mean of 0.96 ± 0.01), indicative of approximately broad band tuning. The distribution also displayed a long tail of discretely clustered cells (Fig. 6A, to the right of the arrow) that we used to define cells with a preference for higher frequencies over low frequencies (1.96 ± 0.3). We then examined the distribution of frequency tuning across all three maps.

When considering the overall frequency tuning characteristics for both bursts and isolated spikes together, we found that the CMS was predominantly low-pass (Fig. 6B) with a small number of broadband cells, previously established to be intermediate E-cells (Ellis et al. 2007b). The CLS cell population was more equitably distributed between low-pass and broadband cells (Fig. 6B), likely reflecting the equal probability of recording from E- or I-cells. Finally, in the LS, approximately half of the cells were low-pass, consistent with approximately half the population being I-cells. The remaining cells were either broadband or high-pass (Fig. 6B). This suggested to us that E-cells of the CMS and LS display specific frequency tuning due to intrinsic conductances that shape firing dynamics and their involvement with low- and high-frequency behaviors, respectively (Metzner et al. 1998; Shumway 1989). In comparison, E-cells of the CLS appear to be intrinsically broadband; this may contribute to the ability of feedback input to rapidly transform their frequency tuning to either high- or low-pass under specific stimulus conditions in vivo (Bastian et al. 2004; Chacron et al. 2003). The different frequency preference across cell type and maps may partially account for the observation that, when using 2- to 40-Hz RAMs in vivo, both E-cells as a population, and the population of LS cells show greater errors in feature detection in comparison to the population of I-cells, or of CMS cells respectively (Metzner et al. 1998). Specifically, grouping these cells using their responses to this frequency range could reveal many of the largest distinctions between cells we observed here, both across maps and between cell classes.

Spike characteristics correlate with frequency tuning

The heterogeneity observed in the frequency tuning of E-cells within maps suggested that frequency tuning may be a more important characteristic to correlate with the spike waveform characteristics rather than the map within which they are located. We therefore compared the spike parameters calculated previously (AHP, threshold, and spike half-width) but grouped the cells by their frequency tuning characteristics rather than by their map of origin. By doing so we in fact found that spike parameters vary most consistently with frequency tuning of the cell, rather than across maps. Low-pass cells showed the shortest spike half-width of any cell class (0.62 ± 0.019 ms), followed by broadband cells (0.73 ± 0.023 ms) with the widest half-widths in high-pass cells (0.98 ± 0.057 ms; Fig. 7A); all populations were significantly different from all others (P < 0.05). We were able to find similar differences in the AHP, where cells belonging to all three categories of frequency tuning were significantly different from each other (low-pass: 6.06 ± 0.26 mV, broadband: 7.19 ± 0.44 mV, high-pass: 9.04 ± 0.60 mV, P < 0.05; Fig. 7B). The threshold for spike initiation in low-pass cells was significantly lower than in broadband or high-pass cells, which were not different from each other (low-pass: −67.0 ± 0.73 mV, broadband: −63.7 ± 0.86, high-pass: −60.6 ± 1.4 mV, P < 0.05; Fig. 7C). We conclude from this that although spike parameters rarely change significantly across maps when cells are grouped purely by map or cell class (see Fig. 4), they do co-vary with frequency tuning of the cells.

Intrinsic gain and frequency tuning

Because of the consistent variation of certain spike waveform characteristics with frequency tuning, we further examined the gain (in Hz/nA) of the firing response as another factor that could influence frequency selectivity. Although not a

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**FIG. 6.** Frequency selectivity is distributed across maps. A: histogram showing the distribution of low-pass, broadband, and high-pass cells. The lower limit for the broadband cells was 0.86 and the upper limit 1.19 (—even cells above this value were considered to display a preference for higher frequencies (e.g., high-pass). Note that for this small sample, the high-pass population occurs as discrete frequency-prefering clusters. The low-pass cells (<0.86) are shown in gray. B: distribution of cell classes across maps. The majority of cells in the CMS are low-pass, with a small number of broadband cells. Cells in the CLS are more equally distributed between low-pass and broadband populations. In the LS, all 3 classes of frequency tuning are observed with low-pass cells making up approximately half of the cells.
parameter of the spike waveform itself, gain is influenced by spike refractory variables, such as the rate of repolarization and AHP (Mehaffey et al. 2005; Troyer and Miller 1997). As with the spike parameters, the gain was homogenous across I-cells of the CMS, CLS, and LS as well as E-cells of the CMS (I-cells CMS: 380 ± 27 Hz/nA, LS: 376 ± 14 Hz/nA, E-cells CMS: 400 ± 20 Hz/nA, P > 0.05; Fig. 8A). Both CLS and LS E-cells were significantly different from all other cell types (P < 0.05) but not from each other (CLS: 270 ± 20 Hz/nA, LS: 186 ± 17 Hz/nA, P > 0.05; Fig. 8A). These distinctions in gain were further accentuated when the cells were grouped by their frequency tuning characteristics, revealing that all cell classes were significantly different from each other with the high-pass cells having the lowest gain (high-pass: 166 ± 22 Hz/nA, broadband: 316 ± 26 Hz/nA, low-pass: 405 ± 21 Hz/nA, P < 0.05; Fig. 8B). These data suggest that one of the outcomes of the differences in spike waveform across different frequency tuning is a variation of gain. This can be readily understood if it is considered that fast firing requires low-frequency inputs as high-frequency time-varying inputs regulate the firing rate independent of the refractory period. In contrast, the firing rate in response to low-frequency inputs depends on the intrinsic refractory period. Thus higher gains encourage low-frequency responses, while a cell with lower gain would be expected to fire fewer spikes in response to low-frequency stimulus components. The lower gain should not affect the response to higher-frequency inputs as under these conditions the limiting factor for the ISIs might be the statistics of the RAM, not the refractory dynamics of the cell.

Adaptation

Another factor that can contribute to frequency tuning is adaptation (Benda and Herz 2003; Benda et al. 2005). We therefore examined the distribution of adaptation rates across cell types and maps. Adaptation was characterized in response to a 0- to 60-Hz RAM stimulus (Fig. 9A) and was well-fit by two time constants. The first, and largest amplitude, time constant was a fast decay between 0.5 and 4 s (representative data and fit is shown in Fig. 9B). The second time constant was far more variable and could either consist of a much smaller, slower decay or display a gradual acceleration. This slow acceleration may be due to the persistent sodium currents previously observed in these cells (Berman et al. 2001; Doiron et al. 2003b). This small magnitude slow adaptation or acceleration could appear in any cell class and could not be consistently related to any of our classifications. We focus primarily on the larger magnitude, fast component of adaptation as it showed a more clear relationship to cell class and across different maps.

We found that a similar time constant for adaptation was apparent in I-cells of the CMS (τ = 2.69 ± 0.24 s), CLS (τ = 2.48 ± 0.30 s), and LS (τ = 2.36 ± 0.10 s), and CMS E-cells (τ = 2.49 ± 0.38 s, P > 0.05). All of these cell types were significantly slower to adapt than E-cells of the CMS (τ = 1.46 ± 0.17 s) and LS (τ = 1.06 ± 0.09 s, P < 0.05; Fig. 9C). When cells were instead grouped by their frequency tuning characteristics, all three groups were significantly different from each other (low-pass: 2.54 ± 0.17 s, broadband: 1.73 ± 0.04 s, high-pass 1.06 ± 0.04 s, P < 0.05; Fig. 9D). We conclude that differences in adaptation are again among the factors that underlie the variability of frequency tuning across ELL maps.

**DISCUSSION**

We have used an in vitro slice preparation to examine the intrinsic cellular properties of two ELL pyramidal cell classes (E- and I-cells) across the three ELL maps receiving tuberous electrosensory input (CMS, CLS, LS). Our primary findings are that 1) the properties of I-cells are relatively homogenous with no significant differences across the maps for any of the...
properties examined, including frequency tuning, 2) E-cells are highly variable across the maps with respect to many of the biophysical parameters examined including frequency tuning, 3) the biophysical differences between E-cells are strongly correlated with their intrinsic frequency tuning. These results are significant in that our data with respect to frequency tuning are concordant with results previously obtained though recordings from ELL pyramidal cells in vivo. Thus I-cells in vivo are generally low-pass in all the tuberous maps while frequency tuning of E-cells transitions from low-pass (CMS) to high-pass (LS) (Chacron et al. 2005; Shumway 1989; R. Krahe, personal communication). Note that results obtained on frequency tuning in vivo are under the influence of many factors beyond intrinsic cellular mechanisms, such as feedback from higher brain centers. Recordings from the in vitro preparation here now show that differences in the intrinsic ion channel contributions to spike properties in E-cells account at least in part for frequency tuning within and across ELL maps in vivo.

Differences across cells, maps, and frequency tuning

The existence of different neural maps that are optimized for distinct types of information processing is a common architecture in sensory systems (Metzner 1999; Schreiner and Winer 2007; Young 1998). In the auditory brain stem, for example, a frequency preference is established in repeating maps that are correlated with specific patterns of channel expression (Brew

FIG. 8. Response gain changes with segment, cell type, and frequency selectivity. A: gain for I- and E-cells of different ELL segments. I-cells have a homogenous gain across segments with no significant difference to CMS E-cells (including broadband cells). In comparison, E-cells of the CLS and LS have significantly lower gain. B: when classified by frequency tuning, the differences in gain become more apparent, gain decreases significantly with higher frequency tuning characteristics. Sample sizes for recordings used to calculate means are shown in brackets.

FIG. 9. The rate of spike adaptation covaries across segments with frequency tuning. A: representative trace of the spike response of a pyramidal cell to a 0- to 60-Hz 100-s RAM intracellular current injection. B: adaptation of spike rate in response to the RAM in the example shown in A. Black lines show the instantaneous firing rate averaged in 1-s bins. The gray line is the fit to the data. C: distribution of adaptation time constants across ELL cell types and segments. E-cells of the CLS and LS have significantly shorter time constants for adaptation than other cell types in other segments. D: adaptation time constant correlates with frequency selectivity with a progressive and significant decrease in adaptation time constant with higher frequency tuning. Sample sizes for recordings used to calculate means are shown in brackets.
and Forsythe 2005; Li et al. 2001; von Hehn et al. 2004). In the ELL, primary afferents from tuberous receptors disperse equally to three distinct spatial maps with similar architectures, yet frequency tuning differs across the three maps. Key differences have also begun to emerge between the E and I subtypes of pyramidal cells in terms of both frequency tuning and ion channel expression (Ellis et al. 2007b). We have now shown that several properties of spike discharge in pyramidal cells are most clearly correlated to frequency tuning. These cells are contained within maps with specific frequency preferences, but spike properties and frequency preferences in pyramidal cells were not perfectly correlated with these maps. This was largely due to the heterogeneity of E-cell as compared with I-cell spike properties and frequency tuning that establishes a differential responsiveness of these projection cells across ELL maps. We would therefore suggest that E-cells should therefore be examined specifically when candidate currents contributing to frequency selectivity are considered. Our results suggest that the conductances underlying the observed differences in spike waveform parameters (e.g., spike half-width, threshold, and AHP), spike threshold and gain, and factors that control pyramidal cell firing patterns (e.g., adaptation and bursting) all contribute to the frequency selectivity displayed by E-cells.

Spike half-width, AHP magnitude, and frequency tuning

Spike half-width and AHP magnitude co-varied with frequency tuning in a map-dependent fashion in E-cells. Both features are likely associated with an increased magnitude and/or duration of the refractory state and might be expected to contribute to frequency tuning, as predicted by a theoretical analysis (Benda and Herz 2003). The refractory variable depends on both Na⁺ channel inactivation and K⁺ channel activation. In fact, Na⁺ channel inactivation and K⁺ channel activation are often grouped into a single refractory variable for simplicity of analysis in some neural models, including this preparation (Fernandez et al. 2005b; Rinzel 1985). We have confirmed that this is the case for SK2 channels, which increase their expression in a mediolateral fashion and contribute to a larger magnitude AHP in E-cells (Ellis et al. 2007b). Indirect evidence suggests that the high-threshold Kv3.1 channel involved in neuronal spike repolarization might also be related to the differences across maps. Expression of Kv3.1 increases from CMS to LS (Deng et al. 2005) and a high-threshold K⁺ channel (possibly of the Kv3 family) has been shown to stabilize high-frequency firing and maintain a brief spike width in ELL pyramidal cells (Fernandez et al. 2005a; Noonan et al. 2003). This suggests that in contrast to SK currents, high-threshold K⁺ currents may be preferentially expressed in I-cells as these cells have a more narrow spike half-width and, compared with E-cells, are capable of higher-frequency firing within each map. Further, these differences between E- and I-cells increase in the more lateral maps, suggesting that an increased Kv3.1 conductance may be contributing to some of the observed differences between E- and I-cells across the maps.

Spike threshold, gain, and frequency tuning

Spike threshold and gain may not intuitively be expected to represent important factors in controlling frequency tuning, but high-frequency-tuned LS E-cells proved to have a higher spike threshold compared with E-cells of the CMS and CLS (Fig. 7C). In this regard, a recent computational analysis has shown that spike threshold, in combination with purely anatomical map differences (e.g., receptive field size), can contribute to frequency tuning (J. Middleton, personal communication). The receptive field size of E-cells increases from CMS to LS (Shumway 1989 for E. virensens; L. Maler, unpublished observation for A. leptorhynchus), and therefore many more P-units converge onto E-cells of the LS compared with the CMS (the CLS is intermediate in this respect). A higher threshold in LS E-cells implies that they require synchronous input from many P-units to reach threshold (J. Middleton, personal communication). Because P-unit discharge is synchronized by high-frequency signals (Benda et al. 2006; J. Middleton, personal communication), this further implies that high-threshold pyramidal cells should be tuned to high-frequency input. The channels responsible for the differences in spike threshold are not known, but one possibility is a differential expression of Na⁺ channels or associated subunits, which, through a shift in either inactivation or activation rates could both raise threshold and increase spike width. Another candidate is the persistent Na⁺ current because this current is prominent in ELL pyramidal cells and enhances excitatory inputs and spike responses (Berman et al. 2001; Doiron et al. 2003b); direct evidence for a differential expression of persistent Na⁺ current in E-cells across maps is, however, not yet available.

Such in vivo effects of spike threshold are likely accentuated by the differential gain of E-cells across the maps. The mechanisms that underlie gain determine the ISI in response to the input rather than the timing of individual spikes. Therefore higher gains are only meaningful for inputs slow enough to generate two or more spikes. A lower gain would be expected to decrease the response to low frequency inputs because fewer spikes would be generated in response to the signal upstrokes. In contrast, the lower gain should not affect the response to higher-frequency inputs as faster oscillations generally create single spikes for each stimulus upstroke (Oswald et al. 2004, 2007). Under these circumstances, the ISI is largely determined by the statistical properties of the time-varying input signal rather than by the gain dynamics of the cell. The biophysical properties determining the intrinsic gain are not completely known, but both subthreshold Na⁺ and spike-initiated Na⁺ and K⁺ channels are likely to contribute (Fernandez et al. 2005b; Noonan et al. 2003).

We have previously shown that the intrinsic gain of pyramidal cells can also be regulated at the network level: dendritic inhibition invoked by a specific interneuron in the ventral molecular layer (VML cell) induces divisive gain control by reducing the DAP emanating from pyramidal cell apical dendrites (Mehaffey et al. 2005). The VML cell receives its input entirely from feedback to the ELL (Maler 1979; Maler and Mugnaini 1994). It is therefore interesting that the LS has the highest density of VML cells (Maler 1979; Maler and Mugnaini 1994; Shumway 1989). This suggests that, more generally, feedback control of gain and frequency tuning (Bastian 1986a,b; Bastian et al. 2004; Chacron et al. 2003; Mehaffey et al. 2005) might be linked through synaptic regulation of the channels responsible for the differential expression of gain across the ELL tuberous maps.
Spike frequency adaptation and frequency tuning

As discussed by Benda and Herz (2003), temporal summation of adapting currents (e.g., currents that lead to a progressive slowing of spike frequency) also contribute to spike frequency adaptation that can promote high-frequency tuning. We have shown that E-cells tuned to higher frequencies show faster adaptation (Fig. 9). These neurons are also those least likely to generate bursts, both in response to DC steps and to time-varying inputs. A faster rate of adaptation is expected to be recruited rapidly during slow upstrokes in the external stimulus by virtue of their generating faster ISIs than those induced by higher-frequency components of the stimulus. A fast firing rate will recruit adapting currents and therefore downregulate the response to low-frequency stimulus components. However, adaptation is unlikely to accumulate as strongly in response to fast stimulus components as these generate primarily single spikes, permitting a more rapid recovery during stimulus-driven pauses in spiking, which in turn allows high-frequency components to be coded more accurately. The biophysical basis of spike frequency adaptation in pyramidal cells is not currently known, but blockade of SK currents with apamin does not prevent adaptation in E-cells (W. H. Mehaffey, unpublished observation), suggesting that another calcium-activated potassium current might be involved. This is consistent with observations in the lateral amygdala where SK currents do not contribute to spike frequency adaptation (Faber and Sah 2002).

Bursting and low-frequency tuning

Many studies have now shown links between burst discharge and low-frequency events in the electrosensory system (Doiron et al. 2007; Krahe and Gabbiani 2004) and in other sensory systems (Krahe and Gabbiani 2004; Lesica and Stanley 2004; Lesica et al. 2006). This appears to be a useful adaptation for generating distinct spike patterns in response to specific features of a sensory signal. In our examination of intrinsic bursting, we found that the distribution of bursting cells varies, occurring most frequently across ELL maps in association with cells that prefer low-frequency events. This included I-cells in all three maps, whereas bursting was less commonly observed in E-cells, particularly in more lateral maps (Fig. 1D). Further, E-cells show lower stimulus-response coherence at low frequencies (0–20 Hz) than I-cells, particularly in the CLS and LS. This is consistent with our previous observations that conductances that can regulate burst threshold are able to decrease the low-frequency component of stimulus-response coherence (Ellis et al. 2007b). In a recent paper, we examined the contribution of an SK-mediated AHP to frequency tuning in response to broadband inputs (Ellis et al. 2007b). This current may make an important contribution to the relationship between AHP and frequency selectivity observed here, but it is interesting to note that although apamin increased the low-frequency coherence in broadband or high-pass cells, it did not alter their high-pass characteristics. This suggests that the SK2 current reduces the low-frequency coherence rather than amplifying high-frequency coherence. Therefore at least one other conductance is likely required to confer high-pass tuning. One candidate current would be the A-type potassium channels that have been described in ELL pyramidal cells (Ellis et al. 2007a; Mathieson and Maler 1988) and that can decrease a neuron’s response to low-frequency input (Ellis et al. 2007a). Further, the A-type channel of pyramidal cells is modulated by muscarinic acetylcholine receptors, and this in turn regulates their frequency tuning (Ellis et al. 2007a). Together this suggests that synaptic inputs can modulate pyramidal cell frequency tuning directly via voltage-gated channels (see preceding text) or via second-messenger regulation of intrinsic conductances.

I-cells and low-frequency tuning

We found that I-cells exhibited low-frequency tuning across all three tuberous maps. The low-frequency tuning observed in vivo (Chacron et al. 2005; R. Krahe, personal communication) is therefore due, at least in part, to the intrinsic properties of I-cells; such as their greater propensity to burst, narrow spike-width and higher gain, allowing a greater response to low-frequency stimulus components. The intrinsic low-frequency tuning of I-cells might also be accentuated by the neuronal architecture of the ELL: because I-cells receive inputs indirectly through the granular cell interneurons, it is possible that this disynaptic relay may contribute to low-pass tuning. It should also be noted that decreases in EOD amplitude (e.g., the stimuli for which I-cells are selective) typically result from the fish swimming past nonconductive objects that generate large low-frequency signals relative to the small amplitude increases in EOD amplitude at the trailing edges of the electric image (Chen et al. 2005; MacIver et al. 2001). Thus the intrinsic and network-dependent tuning properties of I-cells may be well suited to the detection of inanimate nonconductive stimuli such as rocks, although other contexts may lead to large-amplitude decreases in EOD (e.g., beats caused by conspecifics that can contain both high- and low-frequency components).

The vital role of frequency tuning in the ELL is clearly illustrated by the observed contributions at every level of neuronal processing. From the high-frequency selectivity of synchronized presynaptic activity (Benda et al. 2006; J. Middleton, personal communication) to the regulation of conductances that underlie spike generation and firing patterns (Ellis et al. 2007a,b; Fernandez et al. 2005a; Turner et al. 1994) to feedback regulation of frequency tuning by higher brain centers (Chacron et al. 2003, 2005), many factors contribute to regulating the computations performed by ELL pyramidal cells. As we show here, intrinsic cell properties constitute an integral part of the frequency tuning mechanism across sensory maps in the ELL, identifying potential targets for feedback or feedforward mechanisms to regulate cellular properties and thus frequency tuning according to ongoing network activity.

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